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TOWNSEND and TOWNSEND and CREW LLP

By: Malwida Wagit

PATENT
Attorney Docket No.: 20695C-008700US
Client Ref. No.: 27900R1US BX2009T03678

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

I In re application of:

Váradi *et al.*

Application No.: 10/816,099

Filed: March 31, 2004

For: KITS FOR MEASURING THROMBIN GENERATION

Customer No.: 44183

Confirmation No. 9454

Examiner: Rosanne Kosson

Technology Center/Art Unit: 1652

REPLY BRIEF

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This Reply Brief is submitted in response to the Examiner's Answer, dated November 23, 2010, to Appellants' Appeal Brief filed on October 17, 2010.

It is Appellants understanding that no fees are due. However, if fees are due, please charge deposit account no. 20-1430.

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1. STATUS OF CLAIMS

Claims 9, 14-21, and 24 are cancelled.

No claims are allowed.

No claims are objected to.

Claims 1-8, 10-13, 22, and 23 are rejected.

Claims 1-8, 10-13, 22, and 23 are being appealed.

2. GROUNDS OF REJECTION AS SET FORTH BY EXAMINER

In sections 1-7 of the Examiner's Answer Brief, the Examiner had no comments on the Real Party in Interest; Related Appeals and Interferences; Status of Claims; Status of Amendments after Final; Summary of Claimed Subject Matter; Grounds of Rejection to be Reviewed on Appeal; and Claims Appendix as set forth in Appellants' Appeal Brief filed October 17, 2010.

Section 8 of the Examiner's Answer Brief (Evidence Relied Upon) cites the references of record in the rejection of claims 1-8, 10-13, 22, and 23 as allegedly obvious over U.S. Patent No. 6,124,110 to Wöber *et al.* ("Wöber"); in view of U.S. Patent No. 5,625,036 to Hawkins *et al.* ("Hawkins"); Váradi *et al., J. Thromb. Haemostasis* 1:2374-2380, 2003 ("Váradi"); U.S. Patent No. 5,952,198 to Chan ("Chan"); U.S. Patent No. 6,074,826 to Hogan *et al.* ("Hogan"); U.S. Patent No. 6,576,422 to Weinstein *et al.* ("Weinstein"); and U.S. Patent No. 6,756,019 to Dubrow *et al.* ("Dubrow"), which is being reviewed on appeal.

In Section 8 of the Examiner's Answer, the Examiner reiterates various specific grounds of rejection that are of record. Appellants response to the new arguments presented in section 10 of the Examiner's Answer are detailed in section **3. REPLY TO EXAMINER'S ANSWER TO APPELLANTS' ARGUMENTS SET FORTH IN APPEAL BRIEF** below.

In brief, the Examiner characterizes Wöber as disclosing reagents and assays for measuring thrombin generation, including tissue factor (TF) as a dry powder and solutions of phosphatidylserine (PS) and phosphatidylcholine (PC). The Examiner further characterizes Wöber as disclosing the use of TF, PS, and PC to prepare a solution of vesicles containing TF, various ratios of TF to PL in a TF/PL complex; and that the solution may be frozen in assay proportions. Wöber is also cited as disclosing use of a thrombin standard in their assay. Wöber is further described in the rejection as disclosing a dry chromogenic thrombin substrate that is soluble in water, and that the thrombin reaction is initiated by the addition of $CaCl_2$ to the assay samples. Hawkins is cited as disclosing the a TF/PL solution may be lyophilized. The Examiner contends that one of ordinary in the art at the time of the invention would have been motivated to

lyophilize the TF/PL preparation of Wöber because Hawkins teaches that this preparation is made as a reagent for performing prothrombin assays and is meant to be used for large scale clinical assays to have minimal variability.

Váradi is cited in the rejection as teaching a fluorescent thrombin substrate. As noted above, Wöber is described by the Examiner as teaching a dry powder chromogenic that is soluble in water. The Examiner asserts that lyophilized forms of these powders may also be prepared based on Hawkin's disclosure of lyophilized reagents for a clinical assay and further, alleges that one of ordinary skill in the art would have recognized the fluorescent substrate of Váradi is interchangeable with the chromogenic substrate. The Examiner contends that one of skill would have been motivated to prepare a lyophilized reagent containing thrombin substrate and $CaCl_2$ based on Hawkins.

The Examiner's Answer Brief additionally describes the Examiner's rationale for citing Chan, Weinstein, and Dubrow. In particular, the Examiner describes Chan as disclosing liposome of PC, PS, and PE in various ratios. Weinstein is characterized in the Answer Brief as disclosing an assay method using a microtiter plate in which lyophilized detection reagents are immobilized.

3. REPLY TO EXAMINER'S ANSWER TO APPELLANTS' ARGUMENTS SET FORTH IN APPEAL BRIEF

A. The invention

The pending claims relate to a kit, and methods of using such a kit, that has two components: a lyophilized tissue factor (TF)/phospholipid (PL) complex and a lyophilized mixture that contains $CaCl_2$ and a fluorescent thrombin substrate together. Although the mixture is prepared from a solution that includes the fluorescent thrombin substrate, $CaCl_2$, and DMSO, the $CaCl_2$/fluorescent thrombin lyophilized mixture itself has the unexpected property of being dissolvable in water or an aqueous solution without the addition of organic solvent. In the current claims, the amount of water added results in a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$. The invention is based, in part, on the discovery that it is not necessary to add an organic solvent such as DMSO when reconstituting the lyophilized $CaCl_2$/fluorescent thrombin mixture for use. This characteristic is not taught or suggested by the combination of references cited in the rejection, and moreover, as explained below, would not have been expected by a practitioner in this art.

Appellants' arguments in response to the rejection are of record in the Appeal Brief and briefly summarized below. This Brief is in Reply to the Examiner's Answer Brief, in particular, to section 10 of Examiner's paper mailed November 23, 2010.

B. The rejections fails to properly establish the scope and content of the prior art

As previously described, the rejection fails to properly establish the scope and content of the prior art. The fluorescent substrates of the claimed invention are not soluble in aqueous solutions. Further, the combination of references does not teach or suggest a lyophilized mixture comprising both $CaCl_2$ and a thrombin substrate comprising a fluorescent label. Wöber discloses methods of measuring thrombin generation using dried chromogenic substrates, but does not disclose or suggest use of a fluorescent substrate for measuring thrombin generation, much less a lyophilized mixture comprising $CaCl_2$ and the thrombin substrate.

Hawkins describes a prothrombin reagent (PT) containing recombinant tissue factor, natural or synthetic phospholipids, calcium ion, and a buffer (see, Hawkins, col. 2, line 66 to col. 3, line 1). Hawkins indicates that tissue factor-containing reagents should be stable in a lyophilized state (see, col. 1, lines 43-50), but does not disclose or suggest anything regarding the nature of the substrates for a thrombin generation assay.

Furthermore, in contrast to the allegations of the Examiner on page 4, line 22 to page 6, line 22 of the Examiner's Answer to the Appeal Brief, it would not have been obvious to a practitioner in the art to combine Wöber and Hawkins to provide the two lyophilizates characterized in (i) and (ii) of claim 1. Wöber explicitly discloses that phospholipid vesicles containing tissue factor and thrombin are incubated before Ca ions are added (*e.g.*, Wöber col. 2, lines 17-26; col. 5, lines 9-12; col. 6, lines 21-24; the Examiner also noted that Wöber teaches that Ca ions are added to the sample to initiate the reaction). Even assuming *arguendo* that one of skill would have been motivated by Hawkins to lyophilize the phospholipid vesicles containing tissue factor disclosed in Wöber, the practitioner would not have lyophilized a mixture comprising Ca ion and thrombin: Wöber clearly teaches that thrombin and Ca ions may not be added simultaneously to the phospholipid vesicle containing tissue factor, but are always added subsequently to the phospholipid vesicles containing tissue factor with the thrombin being added for a specific incubation time to the phospholipid vesicles containing tissue factor before the Ca ions are added. Accordingly, a person skilled in the art having knowledge of Wöber would have refrained from preparing a lyophilized mixture as defined in (ii) of present claim 1, since dissolving this lyophilized mixture inevitably results in the simultaneous, and not subsequent, addition of thrombin and Ca ions to phospholipid vesicles containing tissue factor. Wöber therefore in fact teaches away from the present invention.

Váradi discloses methods of measuring thrombin generation using fluorescent substrates, which are provided by the manufacturer as a dried powder and reconstituted in a DMSO-containing buffer (explained in Appellants' Brief), but does not suggest a lyophilized mixture that comprises $CaCl_2$ and a fluorescent thrombin substrate or using such a lyophilized mixture in the methods. All of the aforementioned references are completely devoid of any

disclosure or suggestion that would lead to a lyophilized mixture comprising both $CaCl_2$ and a fluorescent thrombin substrate. There is no hint or suggestion in Wöber or Váradi that the thrombin substrates are lyophilized as a mixture with $CaCl_2$. The substrates and $CaCl_2$ are distinct components of the reactions described in each of these references (see, e.g., Wöber at col. 4, line 47 to col. 5, line 40; Váradi at page 2375, second full paragraph).

As explained in Appellants' Appeal Brief, although the Examiner alleges that lyophilisation is an obvious alternative to dry powders and Wöber teaches a chromogenic thrombin substrate that is soluble in water, the rejection fails to discuss the lack of solubility of the fluorescent thrombin substrates in water. The fact that a chromogenic thrombin substrate may be soluble in water is irrelevant to the claimed invention. As explained in the instant specification, addition of $CaCl_2$ to a <u>fluorescent</u> substrate leads to formation of a precipitate (see, e.g., page 9, paragraph 28 to page 10, paragraph 29). Thus, one of skill in the art would not expect that a lyophilized mixture of $CaCl_2$ and a fluorescent substrate prepared as described in the specification could be dissolved in an aqueous solution to form a clear solution. Further, given the insolubility of the recited fluorescent substrate in the absence of an organic solvent, the rejection fails to provide a clear articulation of why one of skill would have made the proposed modification to substitute an insoluble, fluorescent thrombin substrate for a soluble chromogenic thrombin substrate, prepare a mixture with $CaCl_2$ and then lyophilize it with the expectation of obtaining a mixture that is dissolvable in water. No proper evidence or reasoning is provided in the rejection that one of skill would consider this to be a reasonable modification of the cited art.

The disclosures of Chan, Hogan, Weinstein, and Dubrow are not relevant to detection of any protein activity, much less thrombin activity; and are irrelevant to the lyophilized mixtures set forth in the claims for reason of record in the Appeal Brief.

<u>C. The claim elements relating to the solubility recite a physical characteristic of the claimed compositions</u>

The Examiner contends that the limitations set forth in claim 1, part (ii) are obvious. First, the Examiner argues that the wherein clause that recites that the lyophilized

mixture "forms a clear solution when dissolved in water" and that the "amount of water that dissolves the lyophilized mixture to form the clear solution provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$" is not a limitation, but an intended use for the claimed composition. In particular, the Examiner argues that the wherein clause does not materially change the product and the product can be dissolved in any liquid that is compatible with performing a thrombin assay. Appellants respectfully disagree with the Examiner's legal analysis.

In the current claims, the lyophilized fluorescent thrombin substrate/calcium chloride mixture yields a clear solution when it is dissolved in water. This solubility property is a physical characteristic of the compositions. Even though the lyophilized mixture may be suspended in water, saline, a buffer, serum, etc. when the composition is employed in a thrombin generation assay, this does not negate the fact that the composition has the physical property of being readily dissolvable in water.

D. The lyophilized samples described in the Turacek III Declaration were re-suspended in water or a solution that did not contain DMSO.

The evidence provided in the Turecek III Declaration shows that when a lyophilized mixture as set forth in the claims, which contains both the fluorescent thrombin substrate and $CaCl_2$, is lyophilized and re-suspended in water, it is soluble. When just the fluorescent thrombin substrate is lyophilized and the $CaCl_2$ (in water) is used to re-suspend the mixture, it is not readily soluble. The cited art does not lead one of skill to expect this. The Examiner points to Table 1 in the text of the Appeal Brief and alleges that this Table shows that all of the lyophilized samples were re-dissolved in a liquid that yields a final DMSO concentration of 2%. The Examiner's Answer is the first instance during prosecution when this specific assertion was made.

The lyophilized samples referred to by the Examiner are those of the Turecek III Declaration, which was of record during prosecution and provided in the Evidence Appendix of the Appeal Brief filed October 17, 2010. However, Appellants note that Table I was not a part of

the Turecek III Declaration and was not prepared by Dr. Turecek. Table 1 does not correctly reflect the evidence of record in the Turecek III Declaration due to an inadvertent error that was introduced during the preparation of Appellants' response filed February 19, 2009. The methodology employed in the Turecek III Declaration is readily apparent, however, from reviewing the Declaration itself. The Turecek III Declaration and the discussion of the results of that Declaration unambiguously state what solutions were used to dissolve the samples. The samples were dissolved in water or a solution to which DMSO was not added.

Appellants had not noticed the inadvertent error in Table 1and would have corrected it during prosecution had it been pointed out prior to the Examiner's Answer Brief. As this is a new issue not previously introduced during prosecution, it is properly addressed in this Reply Brief. Aspects of the Turecek III Declaration relating to the container in which various experiments were performed and whether the thrombin substrate/$CaCl_2$ is lyophilized with the TF/PL-complex or separately from the TF/PL complex that are of record in the Appeal Brief are not presented again here, as the inadvertent error would have no bearing on this and there is no discussion of these aspects in the Examiner's Answer Brief.

As elaborated in Appellants' Appeal Brief, if one of skill dissolves a fluorescent thrombin substrate in a buffer that also contains 10% DMSO (the fluorescent thrombin substrate Z-Gly-Gly-Arg-AMC.HCL as supplied in powder form must be dissolved in a solution that contains an organic solvent, which is explained in the Appeal Brief) and then adds $CaCl_2$ to that dissolved fluorescent substrate, a precipitate forms. As previously explained, this precipitate is only dissolved with great difficulty such that a clear solution can be obtained. If this clear solution is then lyophilized, this mixture, upon adding sufficient water to provide the recited concentrations of $CaCl_2$ and fluorescent substrate, is clear. This result is unexpected.

As detailed in the Turecek III Declaration and summarized in the Appeal Brief at pages 12 and 13, in the experiments presented in the Turecek III Declaration, different samples were prepared for comparison. Solubility was assessed visually.

In the Turecek III experiments, 250 mg of the fluorescent-labeled thrombin substrate Z-Gly-Gly-Arg-AMC.HCL powder was weighed in a 100 ml flask and initially

dissolved by stirring with a magnetic stirrer in 74 ml of 25 mM HEPES, 175 mM NaCl pH 7.35 buffer, containing 10% DMSO. After the powder was fully dissolved, 6 ml water was added to bring the volume of the substrate solution to 80 ml. The solution remained clear upon the addition of this volume of water. This solution has a substrate concentration of 5 mM in 24 mM HEPES, 175 mM NaCl – 9.25% DMSO buffer. (See, section 5 of the Turecek III Declaration).

The 80-ml substrate solution containing substrate was divided into parts and aliquoted. This procedure resulted in four different substrate samples, two of them lyophilized without $CaCl_2$ and two of them lyophilized after the addition of $CaCl_2$ to the substrate solution. These samples are as follows (Turecek III Declaration, section 6).

Sample 1: Prior to lyophilization, the concentration of substrate in sample 1 was 5 mM with 10% DMSO--**no** $CaCl_2$ is present in the lyophilized sample;

Sample 2: Prior to lyophilization, the concentration of substrate in sample 2 was 2.5 mM, 5% DMSO--**no** $CaCl_2$ is present in the lyophilized sample;

Sample 3: Prior to lyophilization, the concentration of substrate in sample 3 was 1 mM. The solution prior to lyophilization contained 2% DMSO. In addition, the solution prior to lyophilization contained 15 mM $CaCl_2$. $CaCl_2$ **is present** in the lyophilized sample.

Sample 4: Prior to lyophilization, the concentration of substrate, $CaCl_2$, and DMSO is the same as Sample 3; the concentration of the buffer components are different. Sample 4 had the same composition as described in Example 6 (starting at paragraph 50) in the instant specification. $CaCl_2$ **is present** in the lyophilized sample.

During the preparation of the calcium-containing samples for lyophlization, no precipitation was visible upon the addition of $CaCl_2$ dissolved either in water (Sample 3) or in buffer (Sample 4) prior to lyophilization. The solutions containing the thrombin substrate without $CaCl_2$ for samples 1 and 2 were also clear prior to lyophilization (Turecek Declaration, last two sentences of section 6).

The vials were reconstituted and prepared to reflect a "ready to use" solution, containing 1 mM fluorescent substrate and 15 mM $CaCl_2$ in different experiments. Each resolubilization procedure was performed at least in duplicate.

As explained above and on page 13 of the Appeal Brief, in one set of samples of the Turecek III Declaration (samples 1 and 2), the substrate (clear solution) was lyophilized before the addition of $CaCl_2$, such that the solubility of lyophilized substrate without the $CaCl_2$ could be compared to the solubility of the lyophilized substrate mixture that contains $CaCl_2$. The comparative experiments that have the greatest relevance to the current claims are experiments 1a, Experiment 3, and Experiment 4, as explained in the second to the last paragraph on page 13 of the Appeal Brief, as these experiments employ an amount of water to reconstitute the lyophilized preparation that results in the concentration of $CaCl_2$ and fluorescent thrombin substrate recited in the current claims.

Appellants had provided Table 1 in the response filed February 19, 2009 to attempt to simplify the comparison in the Turecek III Declaration; however, as noted above, an inadvertent error was introduced. When Table 1 was created, the "Final Solution Concentration" inadvertently listed all of the ingredients, including DMSO, that had been present in the starting solution prior to lyophilization. However, as evident from the Turecek III Declaration, section 8, none of the solutions used to re-suspend the lyophilized samples contained DMSO.

In Experiments 1a, 3, and 4 presented in the Turecek Declaration, the volume of solution used in resuspending the lyophilized mixture was selected to provide a final concentration of 1 mM fluorescent thrombin substrate and 15 mM $CaCl_2$, as set forth in the current claims.

As explained in the Appeal Brief, last paragraph of page 14, the sample employed in Experiment 1a (Sample 1) had the following concentrations prior to lyophilization: 5 mM fluorescent thrombin substrate and 10% DMSO. The substrate was dissolved and the clear solution was then lyophilized—no $CaCl_2$ was present in the [clear] solution prior to lyophilization and hence, the lyophilized aliquots of this sample. In experiment 1a, an aliquot of this lyophilized mixture was resuspended in 5 mls of 15 mM $CaCl_2$ (no DMSO was included in the re-suspension solution) to provide a solution where the final volume of substrate is 1 mM and the $CaCl_2$ concentration is 15 mM. The substrate was barely soluble (see, section 9 of the Turecek Declaration).

One can directly contrast Experiment 1a with Experiments 3 and 4. For the purposes of comparing concentrations of the fluorogenic thrombin substrate and $CaCl_2$, the discussion in the instant Reply Brief focuses on Experiment 3, but the same observations hold true for Experiment 4. In Sample 3, the concentration of fluorogenic thrombin substrate prior to lyophilization was 1 mM. This solution, prior to lyophilization, also contained 15 mM $CaCl_2$ and 2% DMSO. The thrombin substrate was dissolved and the clear solution was lyophilized. For Experiment 3a, an aliquot was re-suspended in a volume of 1 ml of water. No DMSO was added (section 11 of the Turecek Declaration). This 1 ml of water provides a final concentration of 1 mM substrate and 15 mM $CaCl_2$, as set forth in the claims. The mixture was easily dissolved (Turecek Declaration, section 11a, Experiment 3a; Table 1, column 3). Even if re-suspended in 0.2 ml of water followed by adding HEPES buffer (Turecek Declaration, section 11b, Experiment 3b) the initially opalescent mixture was readily dissolved when the volume was brought to 1 ml with HEPES buffer.

Thus, when the substrate was lyophilized alone and $CaCl_2$ (in water without DMSO) was added as the re-suspending solution, the substrate was not soluble. When the substrate and CaCl2 were lyophilized together and water (again, without DMSO) was added the re-suspending solution, the mixture was readily soluble. Dr. Turecek explains that based on his experience working with fluorescent-labeled thrombin substrates, this was an unexpected finding. He would not have predicted the lyophilized mixture comprising both the fluorescent substrate and $CaCl_2$ to be readily soluble when re-suspended in water.

On page 10, paragraph 2 of the Examiner's Answer Brief, the Examiner acknowledges that Varadi does not teach how the reagents in the thrombin assay are made and that Varadi does not teach a kit.

E. The prior art does not predict the solubility properties of the claimed compositions

The Examiner characterizes Appellants as having asserted that the examiner made an "obvious to try rejection". Appellants respectfully disagree with this characterization of the arguments presented in the Appeal Brief; the point is simply that even if one of skill were

tempted to modify the art as proposed by the Examiner, one of skill could not reasonably have expected that the modifications would successfully result in the current invention.

Part C.1 of the Appeal Brief points out that the rejection does not properly determine the scope and content of the prior art and does not properly explain how the prior lead to the invention as a whole (see, also, page 16 of Appellants' Brief). As further explained on page 16 of Appellants Brief, "In determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." (*Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenk v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983). As explained above, the solubility property of the claimed mixture (in water) is a physical property of the claimed compositions and thus is a characteristic that describes the genus of claimed composition, not merely an intended use. The rejection only establishes that various isolated individual elements (fluorogenic thrombin substrates, the existence of a technique to assay thrombin activity, the existence of lyophilization as a process for preparing various compositions, etc.) were known in the art. Here, the fluorescent thrombin substrate Z-Gly-Gly-Arg-AMC.HCL is known to have only limited solubility in water. One of skill would logically have expected that this would hold true when the substrate is lyophilized together as a mixture with the $CaCl_2$. The inventors discovered that this was not the case.

As discussed above, in the specification, and in the previously submitted Appeal Brief, Dr. Turecek explains in the Turecek III Declaration, the ready solubility of the lyophilized fluorescent substrate/$CaCl_2$ mixture in Experiments 3 and 4 was unexpected.

F. Appellants use of the terms "water" and "aqueous solution" are unambiguous.

The Examiner alleges on page 11 of the Examiner's Answer Brief that it is not clear if lyophilization conditions are such that the DMSO does not evaporate and that whether DMSO was present in the re-suspending solutions used in the experiments described in the Turecek III Delcaration.

As explained above and described in the Appeal Brief and the Turecek III Declaration, the solutions that were used to re-suspend the lyophilized samples in the experiments did not contain DMSO. The solutions were water, or for experiment 4, an aqueous buffer solution that did not have DMSO. Thus, the use of the terms "water" and "aqueous solution" are unambiguous.

The Examiner alleges on page 11 of the Examiner's Answer Brief that it is not clear if lyophilization conditions are such that the DMSO does not evaporate. As previously explained during prosecution and in Appellants' Brief at the last paragraph of p. 15, there is no evidence that DMSO concentration of the solution prior to lyophilization is determinative of solubility after lyophilization. In fact, while Sample 1 contains the highest starting concentration of DMSO (10%) it is the least soluble. In sample 1a, lyophilized substrate without $CaCl_2$, where 5 mL of $CaCl_2$ (in water) was used as the re-suspension solution, the lyophilized Z-Gly-Gly-Arg-AMC.HCL was NOT readily soluble. For sample 3, which prior to lyophilization had 2% DMSO, where the lyophilized substrate Z-Gly-Gly-Arg-AMC.HCL was lyophilized together with the $CaCl_2$, and the solution was resuspended in 1 ml of water (sample 3a) or 0.2 ml of water plus 0.8 mls of 25 mM HEPES/170 mM NaCl (sample 3b), the mixture was soluble. If the solubility characteristics of the lyophilized preparations were simply due to carry over of DMSO upon lyophilization, one would logically have expected the sample that had the highest DMSO concentration prior to lyophilization to be the most soluble. This is not the case. Thus, these data contradict the Examiner's allegation that solubility merely reflects residual DMSO. The data do not support that the starting DMSO concentration is important for the subsequent solubility of the claimed compositions.

On page 12 of the Answer Brief, the Examiner also alleges that the HEPES included in Samples 3b and 4 is a surfactant and would be expected to increase the solubility in water. However, this is irrelevant, as sample 3a was also soluble in water.

The Examiner again notes that she does not consider the wherein clause relating to the solubility of the claimed compositions in water to materially change the product, but rather, to be an intended use. As Applicants have explained, solubility is a physical

characteristic of compositions. The fact that solubility is a physical property of a product does not change because the composition here is a lyophilized mixture. Thus, the element relating to solubility must be considered when determining patentability of the claims.

G. Claims 22 and 23 are separately patentable over the arguments raised in the Examiner's Answer Brief

Appellants further reiterate that the Examiner allegation that the solubility of the claimed lyophilized mixture does not constitute a physical characteristic of the claims is not applicable to method claims 22 and 23. Claims 22 and 23 recite contacting a whole blood or plasma sample with the lyophilized TF/PL-complex and the lyophilized mixture containing thrombin-substrate and $CaCl_2$ and measuring the thrombin generation in the sample. Claims 22 and 23 are patentable for the reasons explained in Appellants' Appeal Brief and for the reasons further explained above. In addition, the claims are separately patentable because the rejection does not establish evidence or reasoning that one of skill could actually expect to successfully perform the claimed method based on the cited art. As explained above, one of skill would not have expected the lyophilized $CaCl_2$/thrombin-substrate mixture to be soluble when an aqueous solution that does not include an organic solvent is used to resuspend the mixture. Therefore, one of skill would not have expected to be able to successfully measure the thrombin generation in the whole blood or plasma sample that is used to dissolve the lyophilized preparation. Claims 22 and 23 are therefore additionally patentable over the art cited in the rejection.

H. Summary

In summary, the solutions used to re-suspend the lyophilized samples in the experiments presented in the Turecek III Declaration are unambiguous. Thus, despite the inadvertent error in Table 1, which was first noted by the Examiner in the Examiner's Answer Brief, the methodology in the Turecek III Declaration and the buffers used for res-suspension can be readily ascertained from the Declaration . Appellants have provided evidence both in the specification and Turecek III Declaration that when a lyophilized mixture as set forth in the claims, which contains both the fluorescent thrombin substrate and $CaCl_2$,is lyophilized and re-

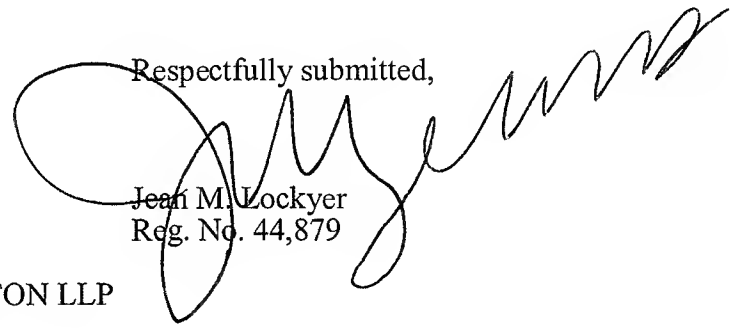
suspended in water, it is soluble. When just the fluorescent thrombin substrate is lyophilized and the $CaCl_2$ (in water) is used to re-suspend the mixture, it is not readily soluble. The cited art does not lead one of skill to expect that the lyophilized mixture would be soluble.

4. CONCLUSION

For the reasons explained in the Appeal Brief and further explained above, the rejection only establishes that some of the isolated individual elements recited in the claims were known in the art, but fails to provide evidence that the current invention, when considered as a whole, would have been expected to result from the combination of references cited by the Examiner. Accordingly, under the standard of obviousness articulated by the Supreme Court in *KSR International Co. V. Telefex, supra,*, the claims are patentable.

In view of the foregoing and Appellants' Appeal Brief, it is respectfully submitted that the rejection be reversed.

Respectfully submitted,



Jean M. Lockyer
Reg. No. 44,879

KILPATRICK TOWNSEND & STOCKTON LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200

5. CLAIMS APPENDIX

1. (previously presented) A kit for measuring the thrombin generation in a sample, said kit comprising

(i) a lyophilized tissue factor (TF)/phospholipid (PL)-complex; and

(ii) a lyophilized mixture comprising $CaCl_2$ and a thrombin substrate that comprises a fluorescent label, where said thrombin substrate that comprises the fluorescent label is Z-Gly-Gly-Arg-AMC; wherein the lyophilized mixture is prepared from a solution comprising the substrate, $CaCl_2$ and DMSO and forms a clear solution when dissolved in water, and further, wherein the amount of water that dissolves the lyophilized mixture to form the clear solution provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$.

2. (original) The kit according to claim 1, wherein the concentration of TF in the lyophilized TF/PL-complex ranges from about 5 to about 1000 pM.

3. (original) The kit according to claim 1, wherein the concentration of PL in the lyophilized TF/PL-complex ranges from about 1 to about 100 μM.

4. (original) The kit according to claim 1, wherein said TF or at least a functional part thereof is of natural or recombinant origin.

5. (original) The kit according to claim 1, wherein said PL is of natural or synthetic origin.

6. (original) The kit according to claim 1, wherein said PL is selected from the group consisting of phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and mixtures thereof.

7. (original) The kit according to claim 6, wherein the weight ratio of PC/PS is in the range of from about 60/40 to about 95/5.

8. (original) The kit according to claim 6, wherein the weight ratio of PC/PS/PE is in the range of from about 60/20/20 to about 78/17/5, based on the total amount of phospholipids.

9. (cancelled)

10. (original) The kit according to claim 1, further comprising at least one thrombin standard.

11. (original) The kit according to claim 1, wherein the lyophilized TF/PL-complex is immobilized onto a support.

12. (previously presented) The kit according to claim 1, wherein the lyophilized mixture comprising $CaCl_2$ and the thrombin-substrate comprising a fluorescent label is immobilized onto a support.

13. (original) The kit according to claim 11 or 12, wherein the support is the inner surface of a vial or wells of an ELISA plate or strip.

14-21. (cancelled)

22. (previously presented) A method for measuring the thrombin generation in a whole blood or plasma sample, comprising the steps of:

(a) providing a lyophilized tissue factor (TF)/phospholipid (PL)-complex and a lyophilized mixture containing a thrombin-substrate that comprises a fluorescent label, where said thrombin substrate that comprises the fluorescent label is Z-Gly-Gly-Arg-AMC, and $CaCl_2$, wherein the lyophilized mixture is prepared from a solution comprising the thrombin substrate, $CaCl_2$ and DMSO and forms a clear solution when dissolved in water, wherein the amount of water that dissolves the lyophilized mixture to form the clear solution provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$;

(b) contacting the whole blood or plasma sample with said lyophilized TF/PL-complex and said lyophilized mixture containing thrombin-substrate and $CaCl_2$; and

(c) measuring the thrombin generation in said sample.

23. (previously presented) The method according to claim 22, wherein the sample is a cell-free plasma sample.

24. (cancelled)